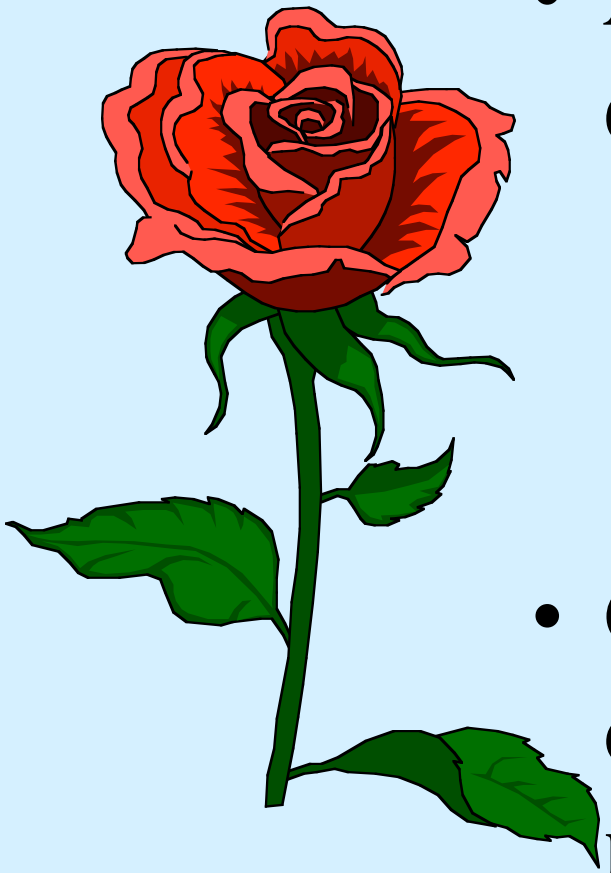
HEALTH EFFECTS OF GM FOOD – WHAT ARE THE ISSUES?

Arpad Pusztai & Susan Bardocz

GENETICALLY-MODIFIED ORGANISMS (GMOS)



- A new technology, with a difference
  - electricity, even nuclear power can be turned off
- GM is self-replicating, cannot be turned off and no method is known to make the gene disappear

ACCORDING TO THE BIOTECHNOLOGY INDUSTRY

- **There is no “credible”evidence that GM crops damage the environment**
- **There is no evidence either that GM food can harm human/animal health**
- **Therefore they are as safe as their “substantially equivalent conventional counterparts” and need no testing**

ARE THESE VIEWS BACKED UP BY PEER-REVIEWED PUBLICATIONS IN SCIENCE JOURNALS?

- **A review concluded that the most pertinent questions on environmental safety of GM crops are just beginning to be studied (Wolfanberger & Phifer, Science, 2000; and ESA Report, Snow et al, 2005)**
- **A review (Domingo, Science, 2000) found only eight peer-reviewed papers published on health aspects of GM food; this increased to a dozen by 2003 (Pusztai et al, 2003) and to 20 by 2005 (Pusztai and Bardocz, 2005)**
- **Royal Society Canada report stated that regulation based on "substantial equivalence" is flawed exposing Canadians to health risks of toxic and allergic reactions**

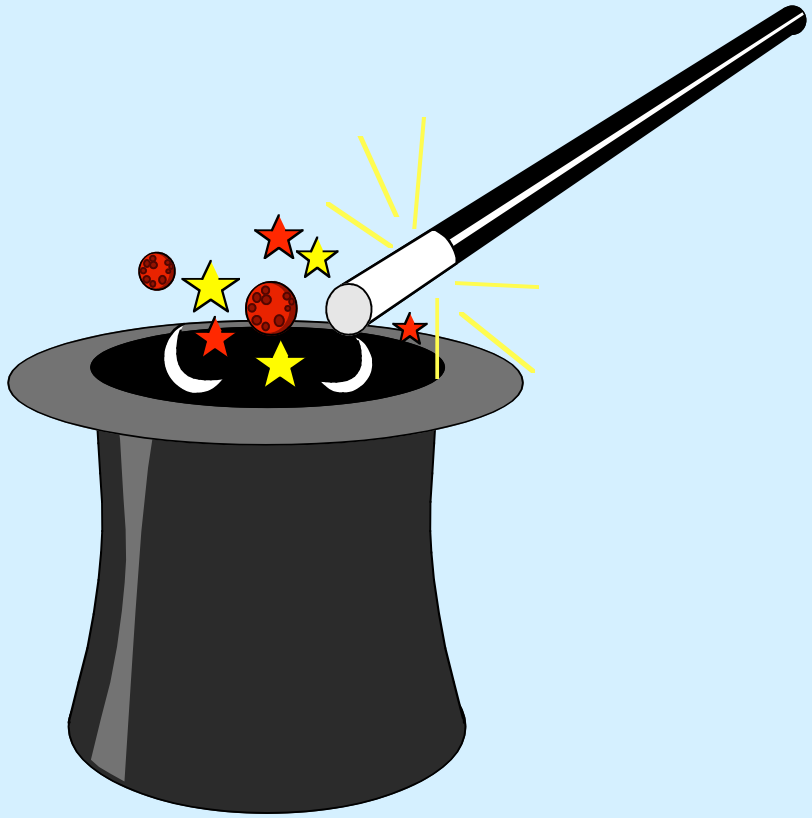
IS IT ACCEPTED THAT GM CROPS SAFE AND NO TESTING IS NEEDED?

- **British Medical Association: Any conclusion upon the safety of introducing GM material into the UK is premature as there is insufficient evidence to decide whether it is safe or not**
- **A majority of British consumers thinks that GM foods are unsafe and don't want to buy them. Thus, UK supermarkets phased them out**
- **European consumers demand labelling of GM and transparent and independent safety testing**

PRESENT STATE OF GM FOOD SCIENCE

- **Many opinions but few data!**
- **Only one human clinical trial and few animal studies have been published to date**
- **The industry's and regulators' preferred "safety assessment" is based on the poorly defined and not legally binding concept of "substantial equivalence"**

HOW CAN A PLANT BE NOVEL AND ‘THE SAME’?



- **The basis of substantial equivalence:**
- **A plant should be novel to be patented (have the new gene)**
- **The GM plant is practically the same as the non-GM, therefore need not be safety tested**

SUBSTANTIAL EQUIVALENCE

- **Similarity in composition is no guarantee that GM- is as safe as conventional food**
- **A BSE-cow is substantially equivalent to a healthy cow**
- **It is a qualitative, non-scientific term; must be used only as a starting point in risk assessment**
- **It must be established by animal testing that GM food has no harmful, toxic/antinutritive or allergenic effects**

SAFETY ISSUES IN GM SCIENCE (NOT DEALT WITH)

- **Methods of plant genetic transformation, role of transgenes, promoters, terminators, selection markers and other construct DNAs, vectors**
- **Establishment of the genomic stability of the GM plant over several generations**
- **Indirect effects on plant metabolism resulting from insertion-site and genome-wide mutations; profiling techniques to detect unexpected changes in the composition of proteins, DNA/RNAs and small metabolites**
- **Ames test to detect mutagens**

TRANSGENE INSERTION (NOT DEALT WITH!)

- **Sequencing the transegene and flanking regions and comparing with that of parental DNA after extensive backcrossing of GM plant**
- **Identifying and discarding GM plants with altered DNA sequences, superfluous DNA insertions, deletions or rearrangements**
- **Identifying insertion sites that lead to aberrant transcripts and/or alter the regulation of neighbouring genes; these plants should also be discarded**

SAFETY ISSUES ADDRESSED IN THIS TALK

- **Selection of "safe" transgene based on short-, long-term and multigenerational animal testing of the gene product before GM transformation**
- **Biological testing of parts of the construct: promoter, terminator, selection markers, reporters, vectors**
- **Exploring direct/indirect effects of GM DNA and proteins on ingestion of GM crops/foods; identifying changes in function, gut-reactivity, immune-, hormonal and metabolic effects**

ALIMENTARY TRACT AS THE FIRST TARGET OF GM FOOD RISK ASSESSMENT

A PERSONAL OPINION OF ARPAD PUSZTAI and SUSAN BARDOCZ

THE CASE FOR BIOLOGICAL TESTING

- **To show the presence of new toxins, allergens, etc by chemical methods is difficult (<u>one cannot determine something that is not known to be there</u>)**
- **In contrast, the consumption of unexpected but potent bioagents can have disproportional large effects on health**
- **Like all foods, GM food will first affect the alimentary tract**
- **FEW EXAMPLES:**


GM food
We bring good things to life.

FLAVR-SAVR^{TM} TOMATO

(see Pusztai et al, 2003)

- **A product of 'antisense' technology**
- **It was claimed that the insertion of Flavr-Savr^{tm} and kan^{r} genes caused no changes in gross fruit composition or the contents of potentially toxic glycoalkaloids**
- **However, daily intubation of normally fed rats with GM tomato homogenates led to serious health problems**

STOMACH EROSION/NECROSIS ON GM AND NON-GM TOMATOES

- **Study 677-004**

- **Non-trg male 0/20**
- **Non-trg female 0/20**
- **Trg male 0/20**
- **Trg female 4/20**
- **re-scored 7/20**

- **Study 677-005 (different tomatoes)**
- **Non-trg male 1/20**
- **Non-trg female 0/19**
- **Trg male 0/20**
- **Trg female 2/15**

EROSION/NECROSIS

- **In humans glandular stomach erosions can lead to life-threatening haemorrhage, particularly in the elderly and patients on non-steroidal anti-inflammatory agents (Pusztai et al, 2003)**
- **Necrosis may also be serious because seven out of forty rats eating GM tomatoes died within two weeks without any explanation**


GM food
It's what's for dinner.

GM POTATOES EXPRESSING BT-TOXIN (Fares & El-Sayed, 1998)

- **Bt-potatoes and Bt-toxin caused the disruption, multinucleation, swelling, increased degradation of ileal surface cells in rats**
- **These effects demonstrated that Bt-toxin survives in functionally and immunologically active form in the gut and had strong effects on gut metabolism**

GM POTATOES EXPRESSING GNA
(Ewen & Pusztai, 1999)

- **Feeding rats GNA-potato-diets induced proliferative growth in their stomach, small- and large intestines and lymphocyte infiltration and suppression of the humoral immune system that was not shown by controls fed non-GM potatoes with or without added GNA**
- **These effects were not due to transgene expression but to its genomic insertion**

RAW GM-POTATO MICROSCOPY (1998 & 1999)

	Fares *et al*	Ewen&Pusztai
Species	male mouse	male rat
Age	4 wk	6wk (100g)
Feeding time	14 days	10 days
Inserted gene	B.thuringensis	galanthus n.ag
Examination	ileal villus planimetry	jejunal crypt image anal.
Result	+21.7%	+57.8%

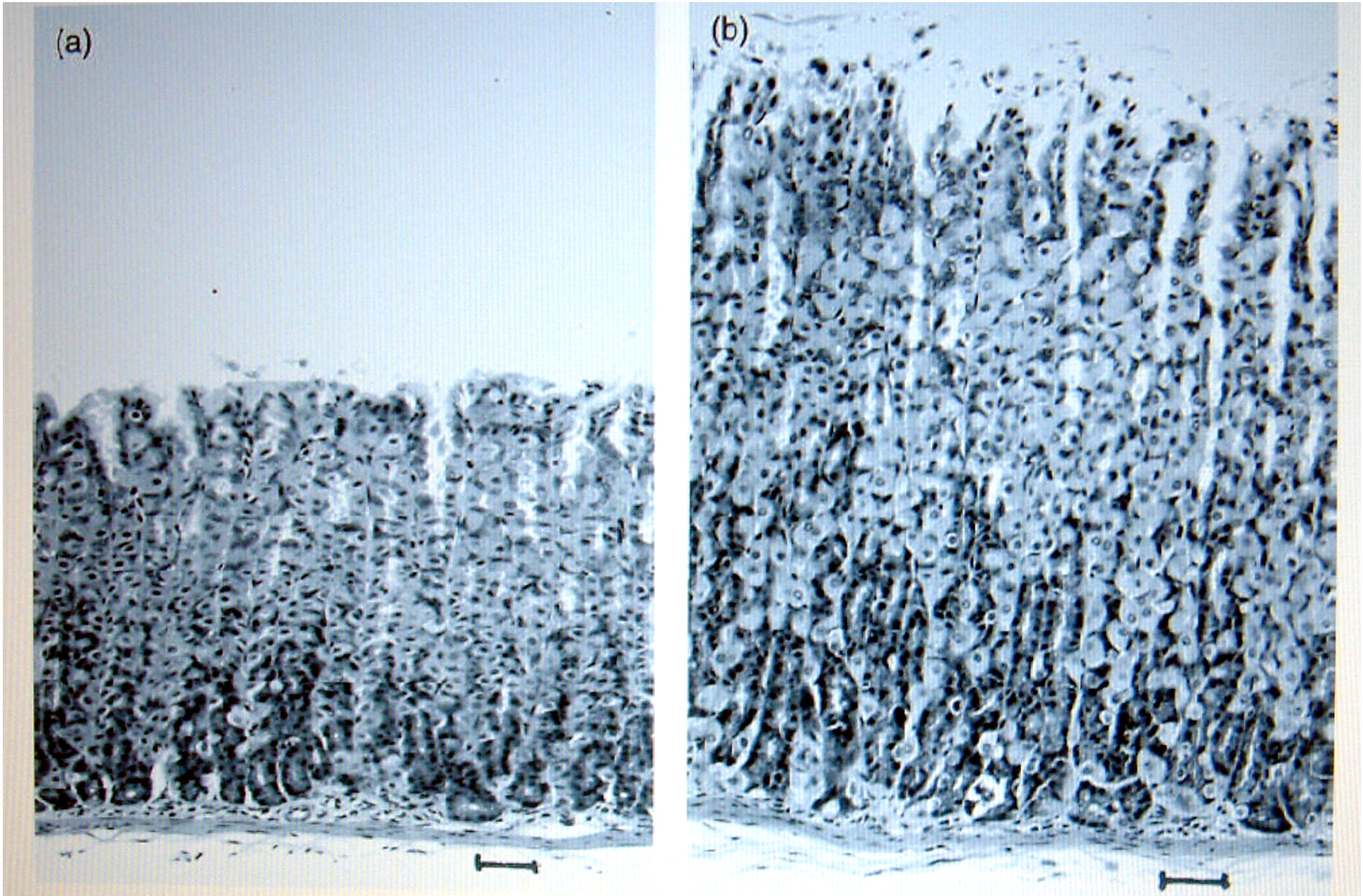
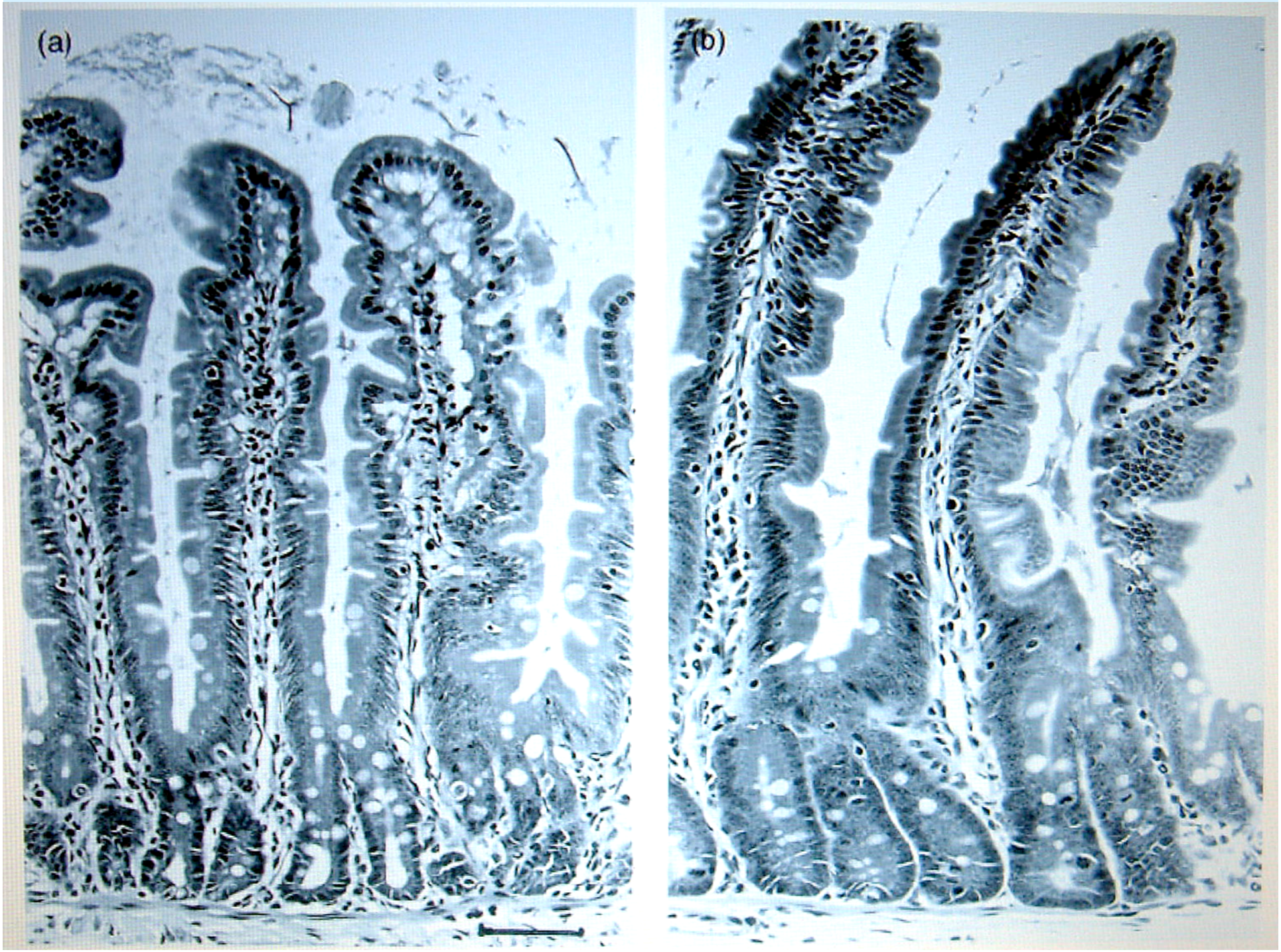


Fig. 16.1. Comparison of the stomach mucosa of rats fed with raw GM potato diet (b) shows marked thickening due to hypertrophy of mucosal cells in comparison with that of rats given the parental line (a) (bar = 100 µm).


(a)
(b)

JEJUNAL CHANGES IN RATS FED GM POTATOES (Pusztai et al. 2003)

	Parent raw	Parent raw +GNA	Raw GM
Crypt cell count	15.8 (1.5)	17.0 (1.6)	20.3 (1.8)
Mitoses (10 crypts)	5.8	5.2	7.5
		$p<0.0005$	$p<0.00001$

IEL /100 ENTEROCYTES - RAW OR BOILED GM/PARENT POTATOES

jejunum	raw	boiled
parent	13.2(2.9)	7.6(0.3)
GNA-GM	21.4(3.9)	10.3(0.3)
	P<0.01	P<0.0001


GM food
It's everywhere you want to be

Bt-CROPS

- **<u>Question</u>: Why people object to the use of *Bt* in GM crops when it has been used in organic farming for decades and nobody objected?**
- **<u>Answers</u>: In *Bt* GM-crops not the bacteria, but the effective part of the bacterial toxin is encoded**
- **In organic farming the bacteria is sprayed only at high insect infestation**
- **Only present on the surface, self-degrades, can be washed off**
- **In the *Bt*-GM crops *every* cell expresses the toxin *all the time*.**

Cry1Ac BINDS TO THE MOUSE JEJUNAL SURFACE

(Vazquez-Padron et al, 2000a)

- ***In vitro*** **indirect immuno-histochemical detection of protoxin binding to fixed jejunal sections**
- **Ligand blotting assay with BBMVs (Brush Border Membrane Vesicles) isolated from mouse small intestine Cry1Ac showed 6 binding proteins**

Cry1Ac IS A SYSTEMIC AND MUCOSAL IMMUNOGEN

(Vazquez-Padron et al, 1999)

- **Both crystalline and soluble Cry1Ac protoxin given intraperitoneally or intragastrically to mice induced high systemic anti-Cry1Ac antibody response**
- **Only the soluble form produced strong mucosal response intragastrically**
- **High antibody levels were detected in the fluids of both small and large intestines**

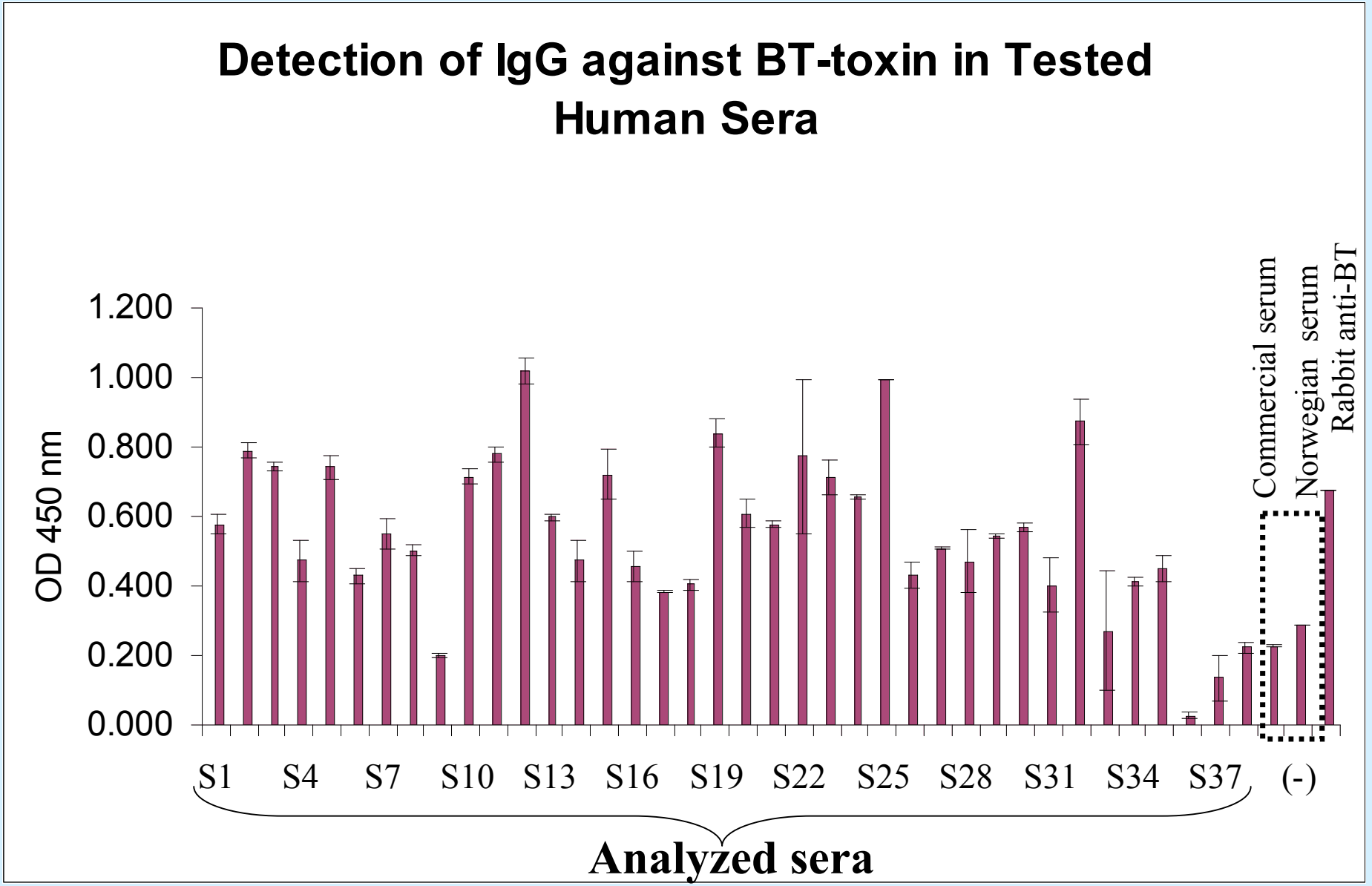
Cry1Ac IS A SYSTEMIC AND MUCOSAL ADJUVANT

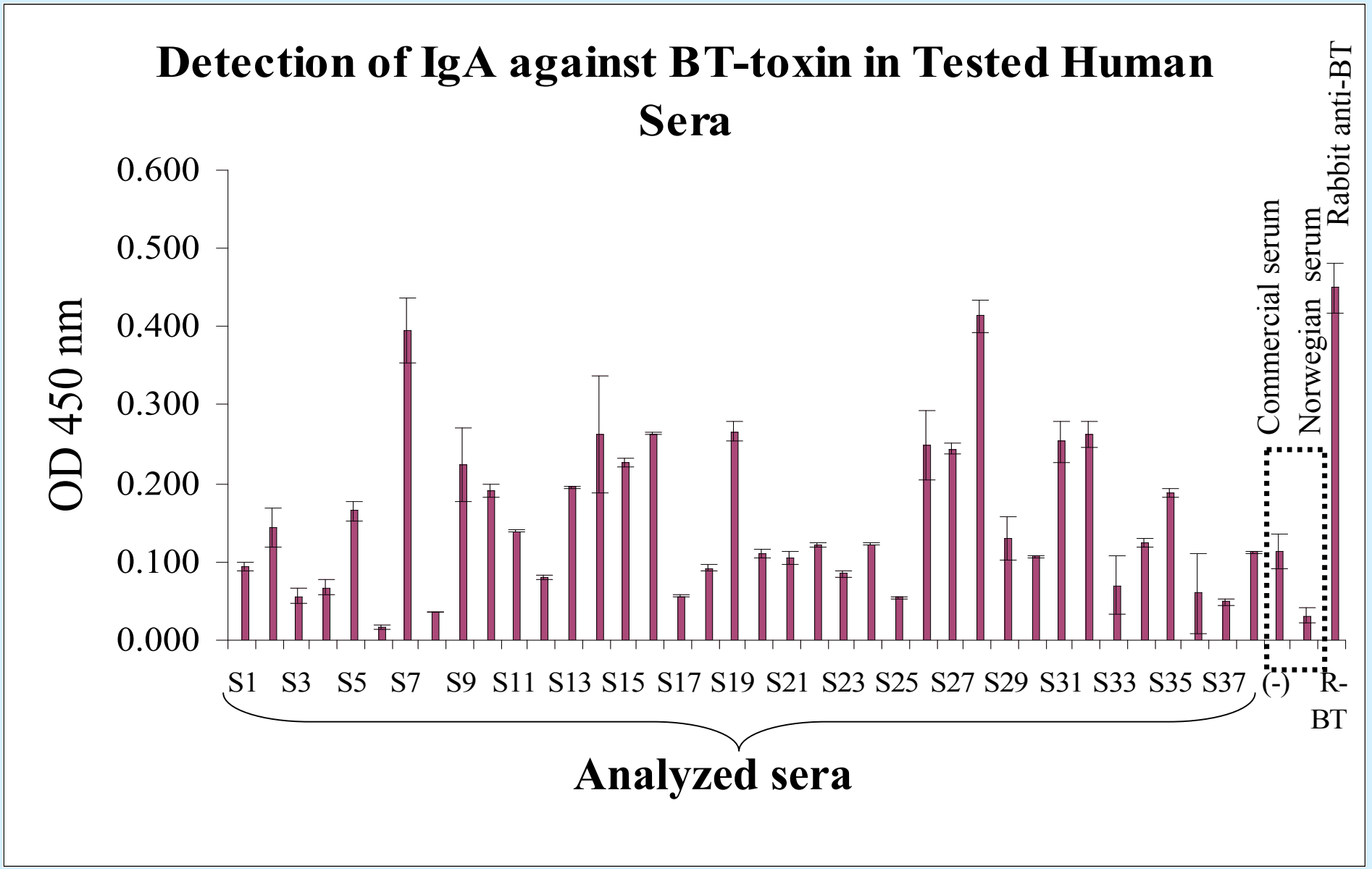
(Vazquez-Padron et al, 2000b)

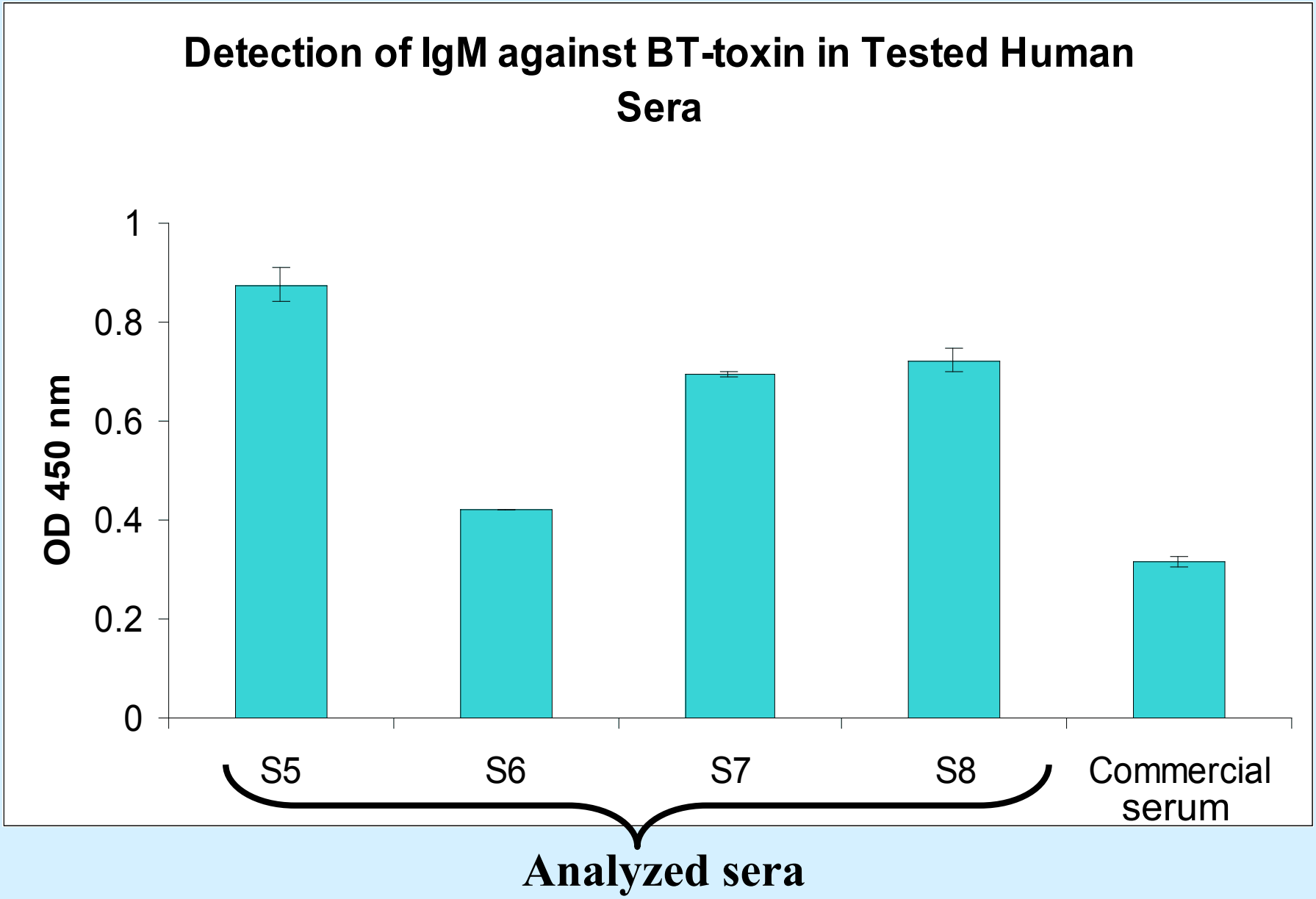
- **On systemic or mucosal co-administration of cholera toxin (CT) and Cry1Ac protoxin together with poor antigens the serum antibody levels to these antigens increased equally.**
- **The enhancement was very strong for serum and intestinal IgG antibody, particularly in the large intestine**
- **Cry1Ac must survive intestinal passage in immunologically active form**

EXPOSURE OF HUMANS TO Bt MAIZE

- **Farmers in the Philippines working on Bt maize (MON 810) fields have developed allergy symptoms which disappeared on moving to other areas but reappeared on return to the same fields**
- **Blood samples taken from these people, when analysed, were found to show Bt toxin antibodies**


Detection of IgG against BT-toxin in Tested Human Sera
OD 450 nm
1.200
1.000
0.800
0.600
0.400
0.200
0.000
S1
S4
S7
S10
S13
S16
S19
S22
S25
S28
S31
S34
S37
(-)
Commercial serum
Norwegian serum
Rabbit anti-BT
Analyzed sera


Detection of IgA against BT-toxin in Tested Human Sera
OD 450 nm
0.600
0.500
0.400
0.300
0.200
0.100
0.000
S1 S3 S5 S7 S9 S11 S13 S15 S17 S19 S21 S23 S25 S27 S29 S31 S33 S35 S37 (-) R-BT
Commercial serum
Norwegian serum
Rabbit anti-BT
Analyzed sera


Detection of IgM against BT-toxin in Tested Human Sera
OD 450 nm
1
0.8
0.6
0.4
0.2
0
S5
S6
S7
S8
Commercial serum
Analyzed sera

INTERPRETATION

- **Specific IgG antibodies in sera suggest that individual has been exposed to antigen, i.e. Bt toxin, during its lifetime.**
- **Specific IgA and IgM antibodies show that the individual has been exposed to the antigen, i.e. Bt toxin, during the last few months.**

IN VITRO SIMULATION OF PROTEIN DIGESTION IN THE GUT IS BASICALLY FLAWED

- ***Assertion*: as GM proteins, such as Bt toxins are digestible in simulated digestibility assays, they will not be toxic or allergenic when eaten!**
- ***Fact*: All lectins resist proteolytic breakdown in the gut *in vivo* but are degraded by proteases in *in vitro* assays and *E. coli* recombinant proteins are quickly degraded both *in vivo* and *in vitro***
- **GM proteins must be isolated from the GM plant. The use in digestibility or toxicity assays of E. *coli* surrogates is unacceptable**

ALLERGENICITY – *IN VITRO* DIGESTIBILITY

- ***Assertion***:
- **All allergenic proteins are indigestible in in vitro digestibility assays**
- ***Fact***:
- **There is no correlation between digestibility measured in vitro and protein allergenicity (Fu et al. 2002)**
- **True digestibility of proteins or DNA can only be established in the gut *in vivo***

ALLERGENICITY IS THE ACHILLES HEEL OF GM (1)

- **<u>Example</u>: GM pea expressing bean α-amylase inhibitor (αAI) gene (Prescott et al. 2005)**
- **Glycosylation and subunit structure of bean and GM pea-expressed αAI were different by Western-immunoblots and MALDI-TOF-MS leading to immunological differences**
- **Bean consumption and respiratory challenge with bean αAI caused no inflammation but that of GM pea led to the development of αAI-specific IgG_1 and footpad challenge of GM pea-fed mice with GM pea αAI led to DTH response**

ALLERGENICITY IS THE ACHILLES HEEL OF GM (2)

- **GM pea-feeding (but not conventional pea) primed mice and when challenged with pea αAI elicited a $CD4^+$ Th_2 cell-mediated inflammation and the production of IL-4 and IL-5**
- **Concomitant exposure of the gut to GM- but not to bean αAI and heterologous food antigens cross primes and elicits immunogenicity**
- **Transgenic transfer of a protein gene from a donor plant species even to a closely related species may lead to the synthesis of structural variants possessing altered immunogenicity**

ALLERGENICITY IS THE ACHILLES HEEL OF GM (3)

- **In skin tests patients reacted differently to GM and non-GM soybeans**
- **GM soybeans contained a unique IgE-binding protein of 25kDa, while non-GM soybeans had a different IgE-binding protein of 30-36 kDa (Yum et al. 2005)**
- **Allergic skin sensitisation to Bt toxin of farm workers (Bernstein et al. 1999) and reports of adverse health effects on aerial spraying with Bt toxin in USA (Carman, 2006 unpublished)**

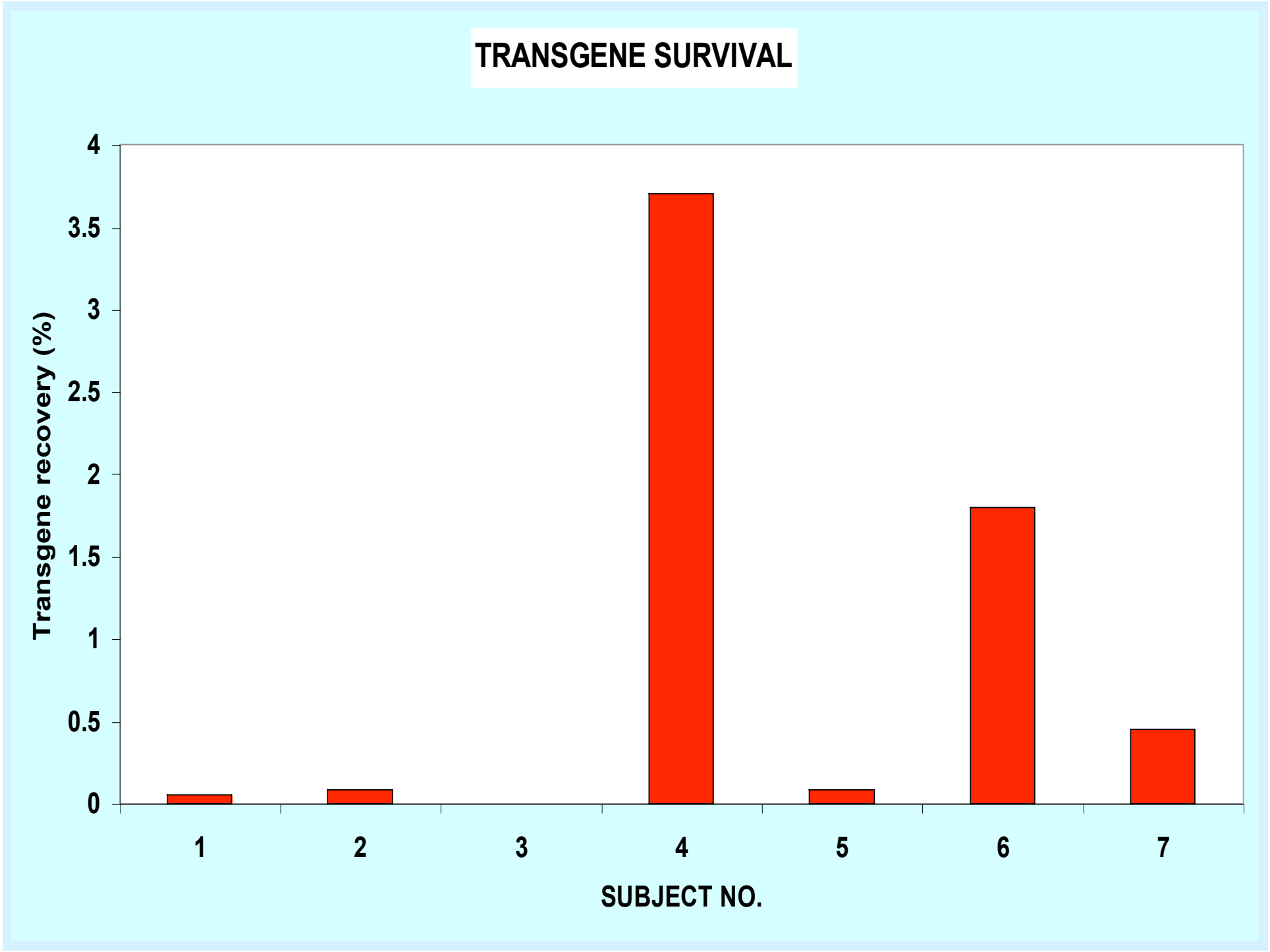
DIGESTION OF DNA

- **Simplistic studies of simulation of *in vivo* digestion in which proteases/DNA-ases are used instead of gastric/intestinal juices are no substitutes for the human situation**
- **Gastric acidity in babies and up to 2/5 of adults is low (high stomach pH); thus DNA/protein survival is higher *in vivo* than suggested by results of *in vitro* assays**
- **This is particularly true for DNA as plant DNA is surrounded by lignin**

PLANT GM DNA AND THE HUMAN GI TRACT

There has been only one human study with GM food (RR soya) to see whether after a single meal the antibiotic resistance marker gene survives in the gut (Netherwood *et al.* 2004)

- **In six out of seven ileostomy patients small but measurable amounts of full length transgene construct was found in the ileostomy bag**
- **3 of 7 ileostomy patients contained CaMV 35s promoter before the study had started**
- **Faeces of 12 volunteer controls contained no CaMV 35s (were they age and sex matched?)**


TRANSGENE SURVIVAL
Transgene recovery (%)
4
3.5
3
2.5
2
1.5
1
0.5
0
1
2
3
4
5
6
7
SUBJECT NO.

TRANSGENE SURVIVAL IN HUMANS

- **The "official" view is that only *small fragments of GM DNA* survived transit while in fact the results showed the presence of *small amounts of full length DNA* in bacteria of the gut pouch**
- **For man all the transgene's important biological effects occur during its gut passage; however its absence from faeces (if true) can benefit the environment**

TRANSGENE SURVIVAL IN PIGS AND RABBITS

- **Fragments of recombinant *Cry1Ab* gene were found in the GI tract, duodenal juice, lymphocytes and liver of Bt11 maize-fed pigs but not in blood (Chowdhury et al, 2003)**
- **Although no GM DNA was found in liver, muscle, kidneys and heart in rabbits fed GM soybean diets (no gut samples!) but significant differences in enzyme levels (LDH) were found in heart and kidneys between GM-fed and control rabbits (Tudisco et al. 2006)**

GM DNA AND PROTEINS IN MILK

- **Although its source is disputed whether from GM feed partially digested in the gut or airborne-, faecal-, or environmental contamination, the presence of GM DNA in milk samples was confirmed (Agodi et al. 2006)**
- **Although its source was similarly disputed the presence of Cry1Ab toxin in milk from Bt maize-fed cows was established (Lutz et al. 2005)**

CONCLUSIONS ON TRANSGENE EFFECTS AND SURVIVAL IN THE GUT

- **The few studies that have been done demonstrate that a great deal of informative data indicating possible major health problems has come from studies of their biological effects on the alimentary tract.**
- **Most interestingly, histological studies of gut sections from GM-fed animals are absent from industry submissions**

HEPATOCYTE NUCLEAR FUNCTION IN GM SOYA-FED MICE (Malatesta et al, 2002a)

- GM soya feeding increases:
- the index of metabolic rate in hepatocyte nuclei
- the number of nuclear pores indicative of intense molecular trafficking
- nucleoplasmic (snRNPs and SC 35) and nucleolar (fibrillarin) splicing factors
- mechanism is unknown

EFFECTS OF GM SOYA ON MURINE LIVER/PANCREAS

(Malatesta et al, 2002b & 2003)

- **Problems: animals were not pair-fed and zone of EM hepatic sample not specified**
- **Nuclei and nucleoli irregular in GM fed suggestive of increased metabolic rate**
- **Reduced digestive enzyme synthesis in pancreas possibly due to reduced post transcriptional hnRNA processing**
- **Soya linked to pancreatic adenoma in rat**

BETA-GLUCURONIDASE

- Steroids, toxins and drugs are detoxified by liver to glucuronide
- Small intestine is almost sterile thus bacterial deglucuronidation is limited
- GUS gene derived β-glucuronidase could amplify deglucuronidation in the small intestine resulting in higher circulating levels of toxins, steroid and drugs

GM CROP HERBICIDE SAFETY

- **(See Pusztai and Bardocz, 2006)**
- **Formulations may cause synergistic, and dose dependent, delay of cells into M-phase**
- **Glyphosate will delay hatching of sea urchin eggs by hatching enzyme inhibition**
- **Glyphosate biocarb increases rat Kupffer cells, deposition of reticulin fibres and increases and leakeage of hepatic transferases and liver damage**
- **Glyphosate toxic to human placental cells at low level (inhibition of aromatase, endocrine disruptor, pregnancy problems, abortion)**

GM DNA SAFETY STUDIES (TROMSO)

- **TASKS:**
- **Trace GM DNA through the intestinal tract**
- **Show whether GM DNA is absorbed into the systemic circulation and body organs**
- **To show whether GM DNA pass into the placenta and foetus?**
- **What are the biological consequences?**

POTENTIAL HAZARDS OF GM FOOD DNA/PROTEIN CONSUMPTION

- **Whether parts of the DNA constructs (containing CaMV 35 s and other helper DNAs) used for gene splicing are taken up by the gut and have biological effects?**
- **Is GM DNA from Bt maize taken up by the gut and has biological effects?**
- **Can the antibiotic resistance gene transform gut bacteria *in vivo*?**
- **Does Bt toxin of GM maize affect the gut, body organs and the immune system?**

BIOLOGICAL RISK ASSESSMENT (1)

- **Assessment of safety of the transgene source**
- **Comparative compositional analysis (profiling) antinutrients, toxins, allergens and metabolites ("substantial equivalence")**
- **Short- and long-term and lifetime feeding trials with young rodents of diets containing the GM plant in comparison with that of the parent line**
- **Evaluation of nutritional value, gut reactivity, effects on hormone-, immune systems and bacterial flora of GM *vs.* parent-line diets**

BIOLOGICAL RISK ASSESSMENT (2)

- **An absolute requirement for the nutritional testing is that all diets must contain the same amount of protein and energy**
- **Two control diets must be used:**
  1. the parent line grown and harvested the same way as the GM
  2. the same control diet to which the gene product isolated from the GM plant is added

BIOLOGICAL RISK ASSESSMENT (3)

- **The growth of groups of pair-fed rats is monitored, and samples of urine and faeces for N- and dry weight balance and blood for immune- and endocrine tests are taken**
- **At the end of feeding the rats are killed, dissected and their gut and other organs are removed for weighing, histology, and DNA and enzyme tests, etc**

BIOLOGICAL RISK ASSESSMENT (4)

- **Statistical evaluation:**
  The *GM food is unsafe* if its effects on rats are significantly different from that of the non-GE parental line control diet
  If the effects of feeding rats with parent line control diet are changed on spiking with the transgene product, the *transgene is unsafe*
  If effects of the GM- and the parent line diets spiked with the gene product differ, the problem is due to *transgene insertion or position*

IDENTIFICATION OF HEALTH EFFECTS WITH MOLECULAR AND CELLULAR EVENTS - SUMMARY

- **Trace through intestinal tract DNA, proteins and metabolites resulting from GM events**
- **Determine consequences of their uptake and biological effects on cells of alimentary tract**
- **Show whether GM DNA and proteins are absorbed into systemic circulation and affect body organs and immune/hormone systems**
- **Show whether GM DNA, proteins and metabolites pass into the placenta, foetus and brain and if so what effects they have?**

PROBLEMS AND PERSPECTIVES

- **Animal tests are but a first step**
- **Next step: multigenerational/reproduction studies with rodents kept on GM food diet**
- **If animal tests showed no harm, GM food safety must be further tested in double-blind, placebo-controlled human clinical studies**
- **It can be expected that harmful effects will be more serious with the old, young and the diseased, particularly those with gut problems**

GM FOOD SAFETY

- **In the absence of safety studies, the lack of evidence that GM food is unsafe cannot be interpreted as proof of its safety**
- **The best way to strengthen the science base of GM food risk assessment is to enlarge the data base by carrying out more work transparently and independent of the industry**
- **The few well-designed studies published to date demonstrate potentially worrisome biological effects of GM food that the regulators have largely ignored**

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